SUPPLEMENTARY INFORMATION

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Supplementary Methods

Cloning, heterologous production and purification of myosin-IE. The expression plasmid pME21 was created by inserting a *KpnI*/*XhoI* fragment from pDXA-Myo1E encoding the 698 residues of the myosin-1E motor domain[1] in the vector pDXA-3FLAG[2]. Serine 334 was mutated to glutamate. *D. discoideum* cells were grown as described previously[3]. *D. discoideum* AX3-ORF$^+$ cells were transformed with the expression plasmids by electroporation[4]. Transformants were selected and grown in the presence of 10 μg/ml G418 (Invitrogen). The myosin-1E motor domain was purified as described previously except for the substitution of the immobilized-metal affinity chromatography step by FLAG-affinity chromatography[1].

Separation of the data set into actin and actomyosin filaments. To identify fully decorated filaments in the data set we employed a two-stage K-means classification scheme. For the first stage we created average images low-pass filtered to ~40 Å of all segment images of each helix and then transformed them into a 1D density profile along the vertical axis. We then applied a 1D mask to remove the central 30 pixels and the outer 60 pixels from the 1D profiles to focus the classification on the region of bound myosin. Filter and mask settings had been determined empirically using a synthetic data set, which had also been used for a prove-of-principle of the approach. Based on the 1D profiles, the data set was split into 16 clusters using a *K*-means algorithm. Of the total 8,367 helices, 5,417 were grouped into three clusters. The remaining 13 clusters were

significantly less populated. To further refine the classification and to visualize the results, we created sub-stacks for each cluster by using the particle and helix IDs to obtain the segment images of the corresponding helices. These substacks (containing standard 2D projection images) were low-pass filtered to 18.5 Å and then each split into 16 clusters using a two-dimensional version of the mask employed in the previous 1D classification. For sub-stacks that did not yield meaningful class-averages this procedure was repeated with either 8 or 4 clusters. Most of the original clusters obtained from the 1D classification contained decorated actomyosin filaments, as judged from the 2D classification class averages and were subsequently combined to form the actomyosin data set. If class averages however were unequivocal, the corresponding particles were removed from the stack. Overall, only few 2D class averages with clearly undecorated filaments were observed.

Correction for the microscope envelope function by scaling of the power spectrum. The aim of this correction step was to enhance higher frequencies dampened by the image formation process of an electron microscope[5]. For this, the 1D rotationally averaged power spectrum of the molecular complex studied was scaled with a power spectrum obtained using a molecular model of the complex. In case of this study, the model was obtained by rigid-body fitting of the known crystal structures [PDB IDs, 3MFP, 1LKX] and the tropomyosin model[6] into the low-resolution electron density obtained. The envelope function effect was ultimately corrected for by adjusting the 1D rotationally averaged power spectrum of the EM structure to that of the model power

spectrum and subsequently the EM structure was low-pass filtered according to the information obtained from the FSC estimate.

Restoration of radial densities by scaling of the radial density profile. To account for a real-space radial fall-off of densities due to misalignment of projection data in iterative refinement of the structure, the 3D reconstructed density map was rescaled according to the radial density distribution of a molecular model of the complex. The same model as used for the power spectrum scaling was employed. To obtain the template radial density profile, the model structure was first averaged to a 2D image along the filament axis and the resulting 2D image was radially averaged. This radial template was then converted to a 3D template cylinder, which was ultimately used for the density fall-off correction. While this restoration had a significant effect on the initial structure determination before separation by codimensional PCA, the effect on the structure determinations of the individual populations was minimal.

Refinement of the contrast transfer function parameters. The CTF determination by CTFFIND3 depends largely on the signal originating from the carbon film surrounding the holey grid. However, the plane of the carbon film does not necessarily coincide. Therefore, the subsequently calculated defocus value for the particles is most likely somewhat different from the actual value. To correct for this, we used the initial CTF calculated for each image as a starting point to obtain refined values using the approach established by Mouche et al.[7]. Briefly, for each micrograph two structures were calculated based on the current estimation of projection parameters. For the first structure

('self') only particles originating from a given micrograph were used. The reconstructed structure was not corrected for the CTF. For the second structure ('other') all other particles were used and the reconstructed structure was corrected for the CTF. Then a Fourier Shell Cross-Correlation Analysis was performed between the 'self' and the 'other' structures, yielding a 1D cross-resolution curve. Zero-crossings of this curve coincide with the zero-crossings of the CTF of the micrograph and can therefore be used to calculate the increased-accuracy defocus setting.

Each 1D cross-resolution curve was evaluated visually and those that did not show clear oscillations were considered too noisy to obtain reliable zero-crossings. In most cases, this was due to a very low number of particles originating from the micrograph used in the reconstruction. For these micrographs the initial defocus values obtained using CTFFIND3 were used instead of the refined values.

Analysis of concerted structural changes induced by complex formation. Analysis on the structural changes associated with formation of the ATM complex was performed using the Bio3d package in R as described for comparative analysis of homologous protein structures[8]. Briefly, we analyzed actin and myosin separately by creating two data sets, one for myosin and actin each. The myosin data set was assembled from the four available models of myoE in the nucleotide bound state[9], actin unbound state (1LKX, chains A-D) and our three models of nucleotide free, actin bound myoE. The actin data set was assembled from the published models of F-actin[10,11] (2ZWH and 3MFP), our undecorated actin model and our three models of actin decorated with myosin and tropomyosin. To make analysis more robust, each model was reduced to alpha carbon

positions only. For each data set we identified an invariable core region by iterative superposition of structures, excluding alpha carbon positions with the highest root mean-square deviation (RMSD) from the mean. The superposed models formed a family of conformers, in which variability of atoms was not restricted to the variability that could be explained readily by the complex formation. To analyze these structural variations in depth, we performed a principal component analysis (PCA) on the atomic coordinates of models within each family. Thus, we computed a covariance matrix $\boldsymbol{C}$ from all pairs x_i, x_j of Cartesian coordinates of atoms in the input models, whose elements are given by:

$$c_{ij} = \left\langle \left(x_i - \left\langle x_i \right\rangle \right) \left(x_j - \left\langle x_j \right\rangle \right) \right\rangle$$

Dominating eigenvectors $\left(\mathbf{e}_l\right)$ of this covariance matrix correspond to major correlated variability of alpha carbon positions about the average structure. We visualized structural changes accounted for by individual eigenvectors by preparing animations of reconstituted structures. The sequence of events corresponds to the average structure modified by the selected eigenvector with the scaling parameter q_l selected such that the variability of the reconstituted structure the variance equal to the variance accounted for by this eigenvector, as given by the associated eigenvalue:

$$\tilde{\mathbf{x}}_l = \left\langle \mathbf{x} \right\rangle + q_l \mathbf{e}_l$$

The resulting visualization is henceforth referred to as a trajectory. The same procedure was performed on subsets of each data set containing either only models of the

uncomplexed proteins or only models of the complexed proteins. Plots were visualized directly in R while trajectories of each significant eigenvector were visualized in Chimera[12].

Supplementary figures

Supplementary Figure 1:

Resolution of the three-dimensional reconstructions of F-actin and the three different conformers of the ATM complex. Fourier shell correlation curves for all reconstructions obtained after conformational analysis by codimensional PCA. According to the FSC = 0.5 criterion all structures had resolutions of approximately 8 Å.

Supplementary Figure 2:

Analysis of structural changes of actin induced by complex formation. (A-F) Variability between the F-actin models obtained from the three ATM complex conformers (group 1-3). (A-B) Visualization of the individual eigenvectors as trajectories of standard deviation scaled displacements from the average model for the first (A) and second (B) eigenvector. (C-D) Contributions of each residue to the first (C) and second (D) eigenvector. (E) Distribution of eigenvalues. (F) Eigenvector-based cluster of the input models. (G-L) Variability between our and published models for bare F-actin (PDB ID: 2ZWH and 3MFP). Panels as for (A-F). (M-T) Variability between decorated (F-actin models obtained from the three ATM complex conformers (group 1-3)) and undecorated (our F-actin EM model and PDB ID: 2ZWH and 3MFP) F-actin models. Panels as for (A-F). Scale bar, 10 Å.

Supplementary Figure 3:

Analysis of structural changes of myosin induced by complex formation. (A-F) Variability between rigor myosin models obtained from the three ATM complex conformers (group 1-3). (A-B) Visualization of the individual eigenvectors as trajectories of standard deviation scaled displacements from the average model for the first (A) and second (B) PC. (C-D) Contributions of each residue to the first (C) and second (D) eigenvector. (E) Distribution of eigenvalues. (F) Eigenvector-based cluster of the input models. (G-L) Conformational differences between pre-power stroke myosin structures (PDB ID: 1LKX chains A-D). Panels as for (A-F). (M-R) Conformational differences between pre-power stroke (PDB ID: 1LKX chains A-D) structures and rigor myosin models obtained from the three ATM complex conformers (group 1-3). Panels as for (A-F). Scale bar, 10 Å.

Supplementary Figure 4:

Model of actin induced force generation in myosin. (A) Overview over structural changes in myosin needed for transition from pre-power stroke (light grey) to rigor state (dim grey). Helices and loops associated with important conformational changes are highlighted in color (bright hue: pre-power stroke position; dim hue: rigor position) and labeled. (B-E) Zooms onto regions mentioned in the text describing the model of the power stroke.

Supplementary Figure 5:

Characterization of the binding interfaces between actin, myosin and tropomyosin. Tropomyosin has been rotated 120˚ clockwise and shifted to the left while myosin has been rotated 120˚ counter-clockwise and shifted to the right. Interfaces were calculated using Intersurf[12] and are delimited by lines drawn onto the surfaces. (A) Overview of the complex. Important loops of actin and myosin are highlighted to help with orientation. (B) Calculated surface hydrophobicity potential using PLATINUM[13]. Hydrophobic patches are colored orange. (C) Calculated surface electrostatic potential at pH 7.2 using APBS[14,15]. Positive-charge density is colored blue and negative-charge density in red. Both tropomyosin and actin appear largely negatively charged, while the interface site of myosin is dominated by positive charges. (D) Surface map colored by residue conservation score as calculated with ConSurf[16]. Conserved residues are shown in pink. Actin and tropomyosin are highly conserved while the interface site of myosin only contains a few conserved residues. Scale bar, 20 Å.

Supplementary Figure 6:

Electrostatic double sandwich between surface loops of actin and myosin. View from the top on the binding interface between actin (back), myosin (front) and tropomyosin (left) colored by electrostatic surface values calculated using APBS[14,15]. The interface between the myosin UD 50 and the actin SD2 domain contains an alternating “sandwich” pattern of strongly charged residues.

Supplementary Movies

Supplementary Movie 1:

Domain flexibility between the three rigor conformations. (A) View along the F-actin axis. (B) View from the F-actin filament onto the binding cleft. The following color code is used to identify domains: UD 50 domain blue, LD 50 domain orange, N-terminal domain green and lever-arm fragment pink. Average domain mass-vectors are depicted as transparent bars. The movie depicts a simple, linear interpolation between the individual ATM groups (3 > 2 > 1 > 3 > 1 > 2 > 3).

Supplementary Movie 2:

Displacement of the lever arm fragment from pre-power stroke position to rigor position occurs along the axis of the F-actin filament. Simple, linear interpolation between pre-power stroke and rigor state of myosin. The lever arm, that would extend from the converter domain in a non-truncated construct is indicated by a red bar to visualize direction of the power stroke.

Supplementary Movie 3:

Domain rearrangement between pre-power stroke and rigor state. (A-D) Views of myosin rotated by 90° around the F-actin axis. The following color code is used to

identify domains: UD 50 domain blue, LD 50 domain orange, N-terminal domain green and lever-arm fragment pink. Average domain mass-vectors are depicted as transparent bars. The movie depicts a simple, linear interpolation between pre-power stroke and rigor state.

Supplementary Movie 4:

Gestalt binding of tropomyosin into a charged groove formed between UD 50 of myosin and SD3 and SD4 of actin. Surface representation colored by electrostatic surface values calculated using APBS[14,15]. The movie zooms in onto the charged groove formed between myosin and actin that accepts tropomyosin in the ATM model.

Supplementary Movie 5:

Tropomyosin shifts between its B and M state. Actin and tropomyosin are depicted in light green and light blue, respectively. The tropomyosin residues R167 are depicted in red.

Supplementary Movie 6:

Shift of tropomyosin along the actin filament between its B and M state. MyoE, which is present in the M state has been omitted in the movie for clarity. Actin and tropomyosin are depicted in light green and light blue, respectively.

Supplementary Movie 7:

Oscillation of tropomyosin between its B and M state can allow initial binding of myosin to actin. Myosin (depicted in red) cannot bind to F-actin (depicted in green) due to steric hindrance by tropomyosin in its B state. Before binding can occur, tropomyosin needs to move to its M state.

Supplementary Movie 8:

Partial rotation of myosin can allow initial binding to actin when tropomyosin is in its B state. Myosin (depicted in red) cannot bind to F-actin (depicted in green) due to steric hindrance by tropomyosin in its B state. However, if myosin rotates by ~ 20° binding can occur even with tropomyosin still in the B state.

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